# The Islamic University of Gaza- Environmental Engineering Department Environmental Microbiology (EENV-2321)

## **Lecture 7: Aquatic Environment**

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Aquatic microbiology can be defined as the study of microorganisms and microbial communities in water environments.

Aquatic environments occupy more than 70% of Earth's surface including oceans, estuaries مصبات الأنهار, rivers, lakes, wetlands, streams, springs, and aquifers.

Microorganisms are key components of the aquatic environment.

As the most important primary producers, microorganisms are responsible for photosynthetically fixing carbon dioxide into organic matter.

Aquatic primary production is estimated to be approximately 50% of all primary production on Earth.

As will be seen, microorganisms are also the most important consumers, responsible for harvesting the organic matter produced through primary production and respiring it back into carbon dioxide

#### MICROBIAL HABITATS IN THE AQUATIC ENVIRONMENT

#### **Planktonic Environment**

Plankton refers to the microbial communities suspended in the water column.

Photoautotrophic organisms within this community including both eukaryotes (algae) and prokaryotes (cyanobacteria) are collectively referred to as phytoplankton العوالق.

Suspended heterotrophic bacterial populations are referred to as bacterioplankton, and protozoan populations make up the zooplankton.

Together these three groups of organisms make up the microbial planktonic community.

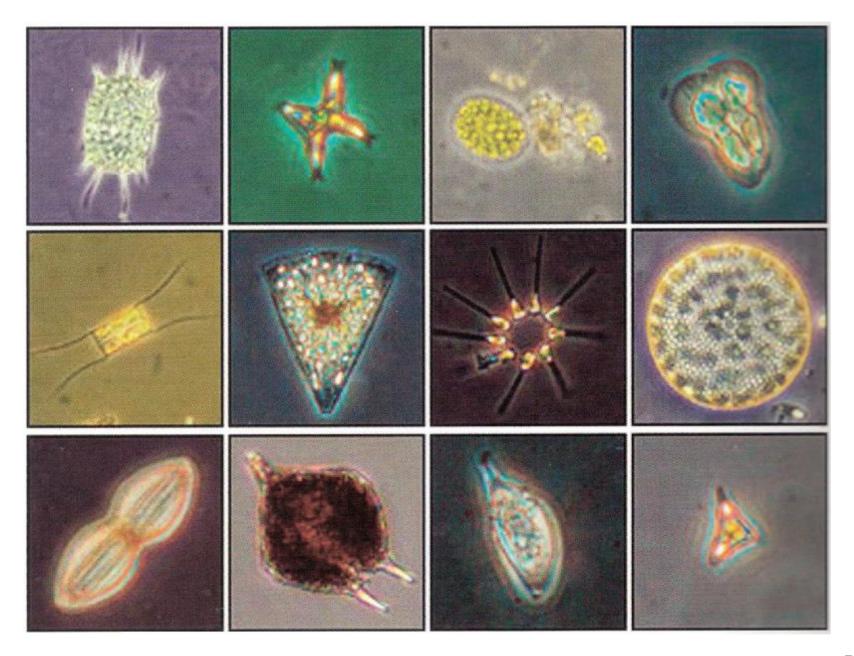
Phytoplankton are the primary producers in the food web, using photosynthesis to fix CO<sub>2</sub> into organic matter.

The organic compounds produced by phytoplankton can be divided into two classes:

Particulate Organic Matter (POM) compounds are large macromolecules such as polymers which make up the structural component of the cell.

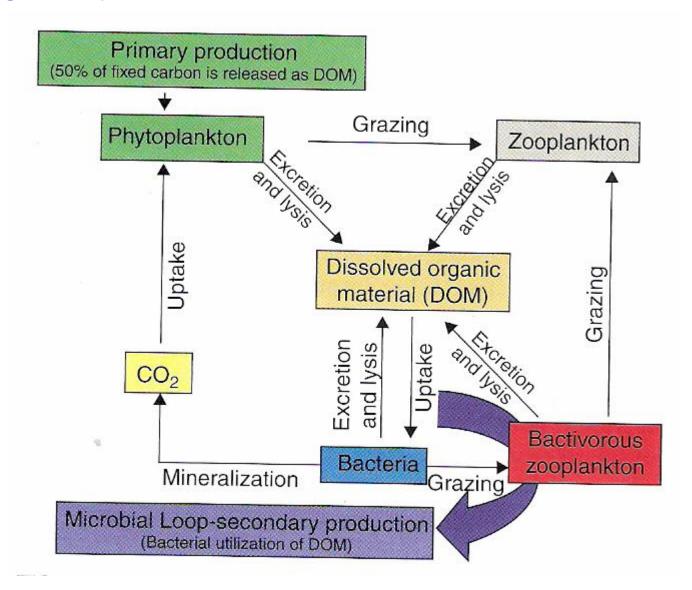
Dissolved Organic Matter (DOM) is composed of smaller soluble material that passes through a filter with a pore size of 0.7µm film including amino acids, carbohydrates, organic acids, and nucleic acids, which are rapidly taken up by microbes and metabolized.

DOM is an extremely large carbon pool, the size of which equals atmospheric CO<sub>2</sub>.



**Examples of phytoplankton** 

The relationship and interdependence of the various microbial components within a general planktonic food web.



## 1. Primary Production

Primary production in the ocean is estimated to be 50 -60 petagrams (1 Pg =  $10^{15}$  g) of carbon per year representing 50% of the total primary production globally.

The amount of primary production within a given water column depends on a variety of environmental factors.

These factors include the availability of essential inorganic nutrient. particularly nitrogen and phosphorus; water temperature, and the turbidity of the water, which affects the amount of light transmitted through the water column.

Open oceans have relatively low primary productivity because of low levels of the essential nutrients nitrogen and phosphorus

The exceptions are areas where currents cause upwelling مياه القاع إلى السطح of water from the bottom of the ocean, bringing with it nutrients from the deep sea

Coastal areas are productive because of the introduction of dissolved and particulate organic material from river outflows and surface runoff from the terrestrial الأرضية environment.

For fresh-water environments, lakes, like the open seas, are often low-productivity environments, particularly those that are large, deep, and nutrient-poor (i.e., oligotrophic).

In contrast, smaller and shallower freshwater bodies tend to be nutrient-rich or eutrophic.

Nutrient loading can be a natural evolutionary process or the result of human activity.

Sources of natural nutrient loading include terrestrial run-off, rivers that feed into the lake, and plant debris such as leaves.

Nutrient loading resulting from human activities includes the disposal of municipal wastewater and runoff of irrigation or rainwater containing fertilizers from agricultural fields.

Both of these nutrient sources contain high levels of nitrogen and phosphorus, the nutrients that are most often limiting in the aquatic environment.

## Runoff recovery systems



## 2. Secondary Production

In a typical food web, phytoplankton (primary producers) are consumed by microfauna (zooplankton), which in turn are consumed by progressively larger organisms, such as fish or other filter feeders.

This is called the grazing food chain. In the open ocean it takes approximately 5 steps or trophic غذائي levels to produce exploitable fish.

The actual transfer of carbon and energy between trophic levels is much more complex than what is implied ضمني by the grazing food chain.

A substantial portion of the carbon fixed by photosynthesis (>50%) is released into the water column in the form of dissolved organic matter.

This DOM is rapidly utilized by heterotrophic bacteria (bacterioplankton), a pathway in the aquatic food web referred to as the microbial loop.

In this loop, bacterioplankton mineralize a portion of the organic carbon into CO<sub>2</sub> and assimilate the remainder to produce new biomass.

This production of bacterial biomass is referred to as secondary production. Secondary production is a major pathway for the utilization of photosynthates, as well as a pathway for the transfer of carbon and energy to higher trophic levels in the aquatic environment.

Thus, the microbial loop serves to efficiently utilize the DOM released into the water column.

The DOM pool that is available for secondary production comes primarily from phytoplankton, with contributions from zooplankton and bacterioplankton.

Aquatic fauna contribute to this pool as well through excretion and the lysis of dead cells.

Among the phytoplankton, it is known that both "healthy" cells and "stressed" cells (those under some form of environmental stress) can release DOM into the water column.

Another suggested mechanism is that "sloppy" feeding habits of aquatic animals الحيوانات المائية that prey تقتات on phytoplankton may allow a portion of the DOM to be released into the water column.

Finally, evidence indicates that as much as 6 to 26% of DOM is released during the lysis of phytoplankton and bacterioplankton by viruses

### **Benthic Habitat**

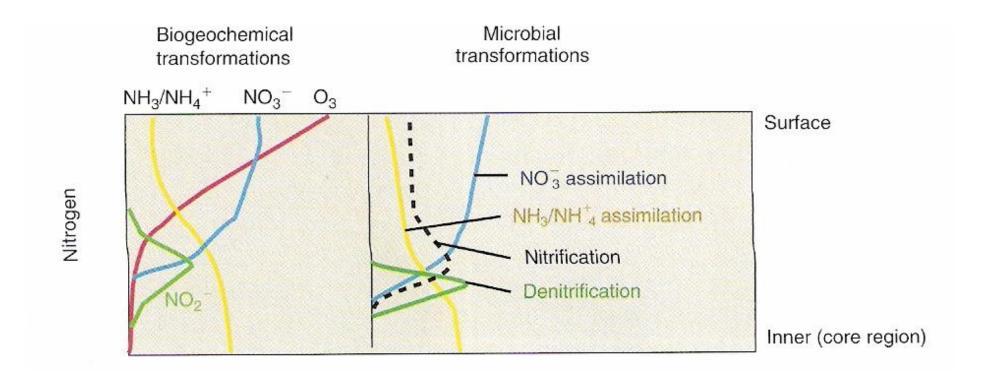
The benthos is a transition zone between the water column and the mineral subsurface.

This interface collects the organic material that settles from the water column or that is deposited from the terrestrial environment.

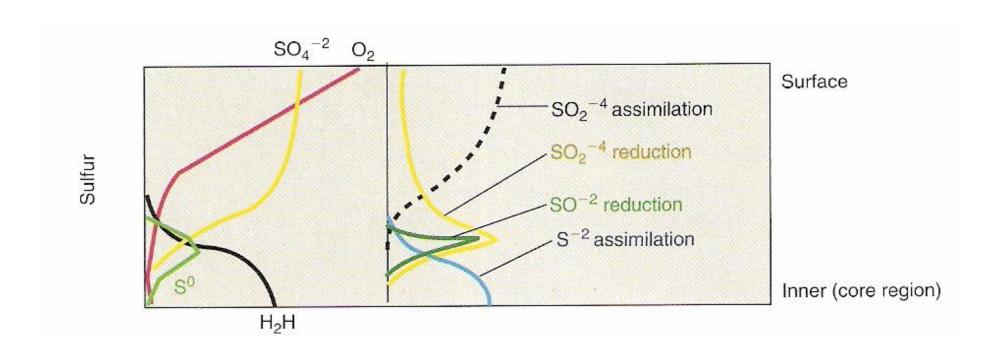
Because nutrient levels are increased, this zone is characterized by a dramatic increase in microbial numbers (as much as five orders of magnitude) and activity compared with the planktonic environment.

Since activity is high, oxygen is utilized quickly and as a result, the benthic environment supports aerobic and anaerobic micro-environment.

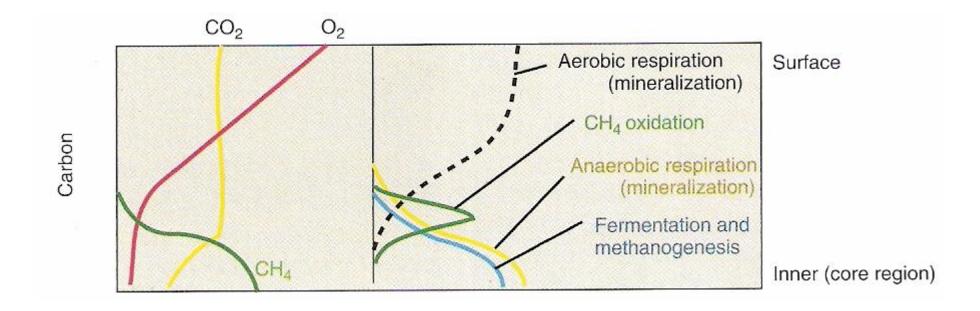
Biogeochemical profiles and major Nitrogen transformations that can be predicted for benthic environments in which oxygen levels are highest at the surface layer and are depleted by microbial activity to create anoxic condition in the inner region



Biogeochemical profiles and major Sulfur transformations that can be predicted for benthic environments in which oxygen levels are highest at the surface layer and are depleted by microbial activity to create anoxic condition in the inner region



Biogeochemical profiles and major Carbon transformations that can be predicted for benthic environments in which oxygen levels are highest at the surface layer and are depleted by microbial activity to create anoxic condition in the inner region



## **Microbial Mat**

Microbial Mats, on the other hand, are specialized microbial communities composed mainly of photosynthetic procaryotes.



The principle distinction between microbial mats and other biofilms is their dependence on photosynthetic primary productivity as their source of energy

## **Biofilms**

Biofilms are composed of populations or communities of microorganisms adhering to environmental surfaces.

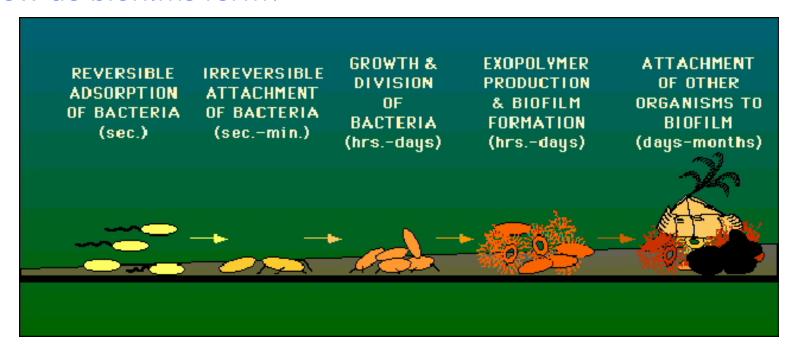
They are usually encased in an extracellular polysaccharide that they themselves synthesize.

Biofilms may be found on essentially any environmental surface where there is sufficient moisture.

Their development is most rapid in flowing systems where adequate nutrients are available

Typical locations for biofilm production include rock and other substrate surfaces in marine or freshwater environments.

#### How do biofilms form?

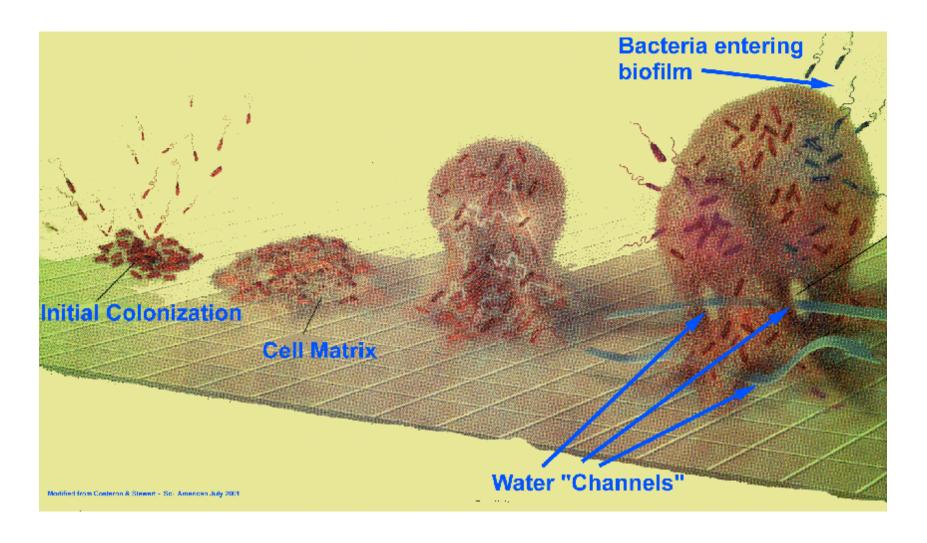


- 1. Typically, within minutes, **an organic monolayer** adsorbs to the surface of the slide substrate. This changes the chemical and physical properties of the glass slide or other substrate.
- 2. These organic compounds are found to be polysaccharides or glycoproteins. These adsorbed materials condition the surface of the slide and appear to increase the probability of the attachment of planktonic bacteria

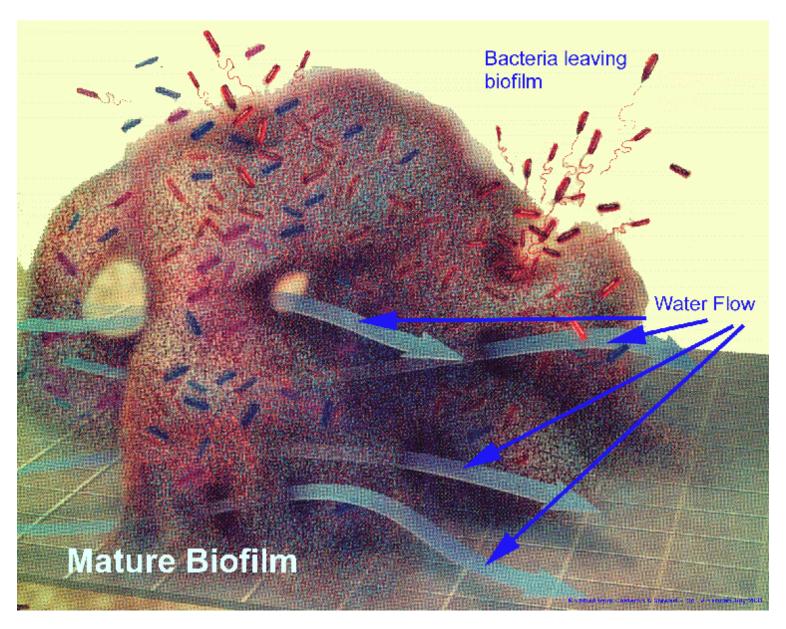
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- 3. Free floating or planktonic bacteria encounter the conditioned surface and form a reversible, sometimes transient attachment often within minutes.
- 4. This attachment called **adsorption** is influenced by electrical charges carried on the bacteria, by Van der Waals forces and by electrostatic attraction although the precise nature of the interaction is still a matter of intense debate. In some instances, as for example, in the association between a pathogen and the receptor sites of cells of its host there may be a stereospecificity which though still reversible is stronger than that achieved strictly by ionic or electrostatic forces.
- 5. If the association between the bacterium and its substrate persists long enough, other types of chemical and physical structures may form which transform the reversible adsorption to a permanent and essentially **irreversible attachment**.

- 6. The final stage in the irreversible adhesion of a cell to an environmental surface is associated with the production of Extracellular Polymer Substances or EPS. Most of the EPS of biofilms are polymers containing sugars such as glucose, galactose, mannose, fructose, rhamnose, N-acetylglucosamine and others.
- 7. This layer of EPS and bacteria can now entrap particulate materials such as clay, organic materials, dead cells and precipitated minerals adding to the bulk and diversity of the biofilm habitat. This growing biofilm can now serve as the focus for the attachment and growth of other organisms increasing the biological diversity of the community.

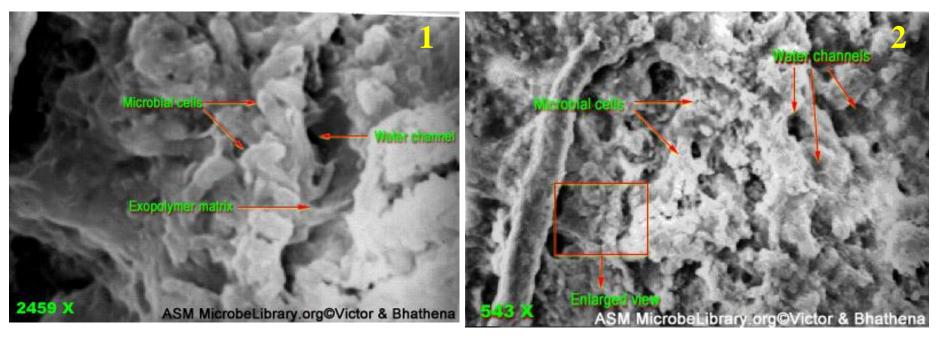


Colonization and adsorption to a surface are followed by the matrix production and development of the water channels.



A mature biofilm in a flowing environment may lose bacteria to the surrounding water.

## **Biofilm Development in a Water Supply Pipe**



These images show the colonization of opaque — surfaces in a water supply by a heterogeneous microbial population in a nutrient limiting environment using scanning electron microscopy.

Figure 1 shows the surface topography of the biofilm present on a galvanized iron surface (water pipe line material) at a magnification of 543X.

Figure 2 shows microbial cells, exopolysachharide material and water channels present in the boxed area from Figure 1 at 2459X magnification.

#### **AQUATIC ENVIRONMENTS**

#### 1. Freshwater Environments

Freshwater environments, such as springs, rivers and streams, and lakes, are those not directly influenced by marine waters.

The science that focuses on the study of **freshwater** habitats is called **limnology**, and the study of freshwater microorganisms is microlimnology.

There are two types of freshwater environments:

Standing water, or lentic habitats, including lakes, ponds, and bogs المستنقعات

Running water, or lotic habitats, including springs, streams, and rivers.

These freshwater environments have very different physical and chemical characteristics and correspondingly different microbial communities and activities.

For instance, the microbial community in a lake in Egypt is not the same as the microbial community in one of the Great Lakes in the northeastern United States.

## a. Springs

Springs form wherever subterranean water reaches Earth's surface.

Microorganisms, especially bacteria and algae, are often the only inhabitants of springs.

In general, photosynthetic bacteria and algae dominate spring environments, with communities ranging from 10<sup>2</sup> to 10<sup>8</sup> organisms/ml.

These primary producers are present in highest concentrations (10<sup>6</sup> to 10<sup>9</sup> organisms/ml) along the shallower edges of the spring and in association with rock surfaces, where light is available and inorganic nutrients are in highest concentrations

Although heterotrophs are also present, numbers are usuall low (10<sup>1</sup> to 10<sup>6</sup> organisms/ml) because DOM is low.

As they mature and die, photosynthetic populations provide the initial source of organic matter for downstream heterotrophic populations.

However, the largest portion of DOM found in surface freshwater originates from surrounding terrestrial الأرضية sources.

This organic input, which originates from sources such as plant exudates, dead plants, animals, and microbial biomass, is transported into lotic habitats by mechanisms such as terrestrial runoff, seepage, and wind deposition.

Thus, we have the image of spring water starting at its source with very low concentrations of DOM and heterotrophs.

The DOM and the heterotrophic populations steadily increase as the spring moves away from the source and as inputs of terrestrial organic matter and microbial bio-mass continue to accumulate.

#### b. Rivers and Streams

Springs, as they flow away from their subsurface source, merge with other water sources to form streams and rivers that eventually flow into other bodies of water such as lakes or seas.

Streams contain primary producer communities, especially when light can penetrate to the bottom of the stream.

Photosynthetic populations range from 10<sup>0</sup> to 10<sup>8</sup> organisms/ml and tend to be present as attached communities associated with biofilms because of the flowing nature of the water column.

Phytoplankton (free-living) communities also exist in streams, but because of the constant water movement, they are not spatially stable populations.

As a stream progresses and becomes larger, it tends to accumulate DOM from surface runoff and sediments.

The increase in DOM limits the penetration of light and consequently begins to limit photoautotrophic populations.

In turn, heterotrophic populations begin to increase in response to increased DOM.

In general, the concentration of heterotrophs in streams and rivers ranges from 10<sup>4</sup> to 10<sup>9</sup> organisms/ml, with microbial numbers increasing as DOM increases.

A measurement of virus to bacterium ratios (VBRs) in the Danube River, Germany, gave an approximate range of 2-17.

Because of their flow patterns, stream and river waters are for the most part well aerated. Therefore, heterotrophic populations are predominantly aerobic or facultative aerobic. Sewage outflows are areas where this is especially evident. Downstream of these outflows, heterotrophic populations often increase two to three orders of magnitude.

Although isolated pools that form in rivers act as DOM and POM sinks and support fairly stable heterotrophic planktonic communities, the only truly stable populations in the lotic habitats of streams and rivers are the biofilm and sediment (benthic) communities.

#### c. Lakes

## **Physical and Chemical Characteristics**

Lakes vary in depth from a few meters to more than 1000m.

Lakes can also vary considerably in surface area, from small ponds of only a few square meters to large lakes that cover areas of up to 100,000 km<sup>2</sup>.

Although often regarded as lentic or non-flowing environments, lakes have inflows and outflows, wind-generated turbulence, and temperaturegenerated mixing, all of which create a dynamic environment.

Salt lakes, such as the Great Salt Lake in Utah, are distinguished by a high salt content and are examples of extreme environments.

Other lakes are also characterized by chemical composition, such as bitter lakes that are rich in  $MgSO_4$ , borax lakes that are high in  $Na_2B_4O_7$ , and soda lakes that are high in  $NaHCO_3$ .

Lakes are by far the most complex of the fresh-water environments. As a result, the microbial communities and their interactions are equally complex and diverse.

Lakes are divided into subsections based on morphometric (depth, dimension, geology of shores, currents, etc.) and physicochemical (temperature, pH, oxygen content) parameters

The edge of the lake, where sunlight can penetrate to the bottom, is known as the littoral zone.

The air-water interface including the upper few millimeters of the water column is known as the neuston layer.

The neuston is known to accumulate nutrients. The very top layer of the neuston is a thin lipid layer (I0 nm deep) that is created because it is energetically favorable for non-polar organic molecules to align at the airwater interface

Adjacent to this is a slightly thicker layer (100 nm) containing proteins and polysaccharides.

Together, these layers form a thin gel-like matrix at the air-water interface.

Bacteria attach to this organic layer in a firm (dense) but reversible manner. Thus, we also have the image of the neuston layer as a biofilm, where organic molecules "condition" the air-water interface, allowing bacteria to attach.

The limnetic zone refers to the surface layer of open water away from the littoral zone where light readily penetrates.

The area below the limnetic zone, where light intensity is less than 1 % of sunlight (the light compensation point), is known as the profundal zone.

Finally, the benthic zone consists of the lake bottom and the associated sediments.

Temperature is very important, especially in lakes, giving rise to another classification scheme.

The three regions in this scheme are the upper zone or **epilimnion**, the lower zone or **hypolimnion**, and the **thermocline**, which is a middle zone characterized by a rapid change in temperature.

Because water is most dense at 4°C, temperature-induced density stratification occurs at the thermocline in the summer and the winter.

In the summer, the epilimnion, which is heated by sunlight, is typically warm and oxygen rich.

This zone is usually characterized by intensive primary productivity that can deplete the epilimnion of mineral nutrients, resulting in nutrient-limiting conditions.

The characteristics of the epilimnion are reversed in the hypolimnion, which has low temperature and oxygen levels, lack of light penetration, and a high mineral nutrient content.

This stratification would tend to make lakes very static, but as fall and winter approach, the warm waters of the epilimnion cool until they reach the temperature, and consequently the density, of the hypolimnion.

When this happens the thermocline breaks down and allows mixing of the epilimnion and the hypolimnion.

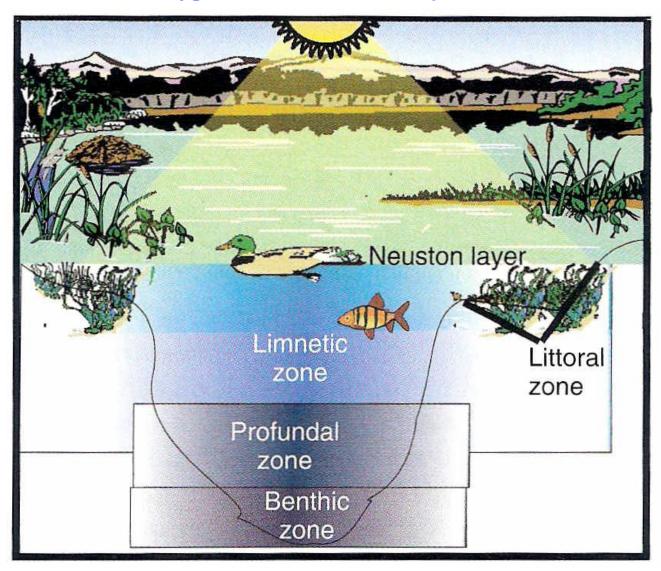
In the winter a layer of ice forms at the top of the lake and the epilimnion is formed in the region of  $O^{\circ C}$  (ice layer) to  $4^{\circ}C$ .

The hypolimnion remains at 4°C or warmer, and again a thermocline is formed and no mixing occurs.

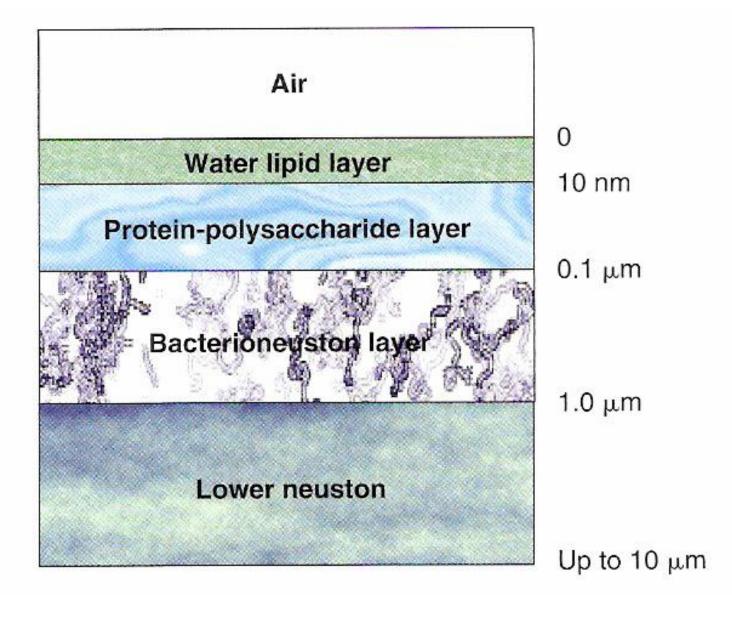
In the spring, as the lake thaws and the two zones reach a similar temperature, mixing occurs once again.

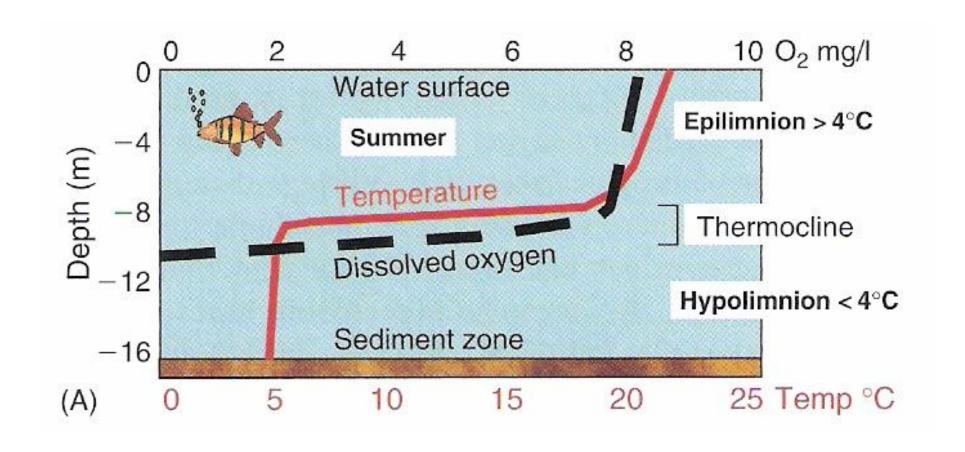
The turnover and mixing of these two layers allow reoxy-genation of the hypolimnion and replenishment of mineral nutrients in the epilimnion.

Schematic representation of a typical lake showing common designations based on sunlight. Other designations for zones are based on features such as temperature, oxygen concentration, and pH.

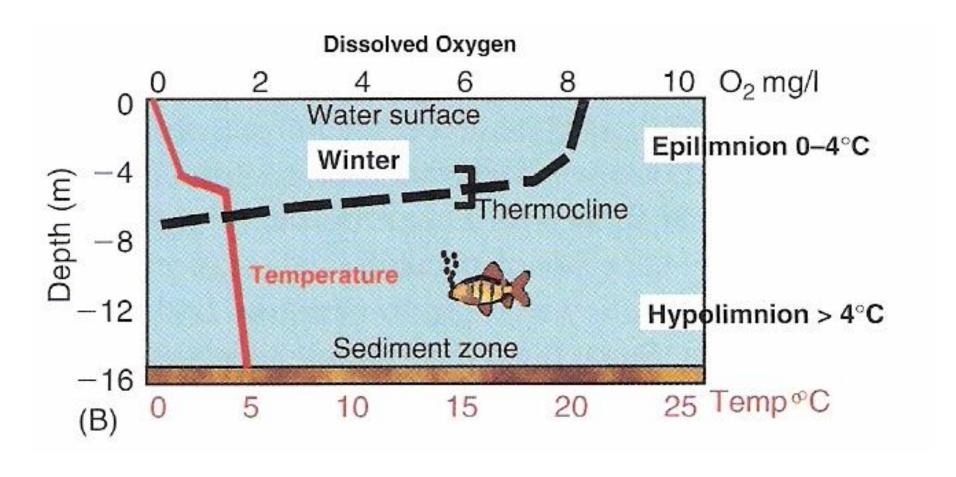


# Schematic representation of the neuston. This is the upper layer of aquatic environments and can range from 1 to 10 µm in depth.



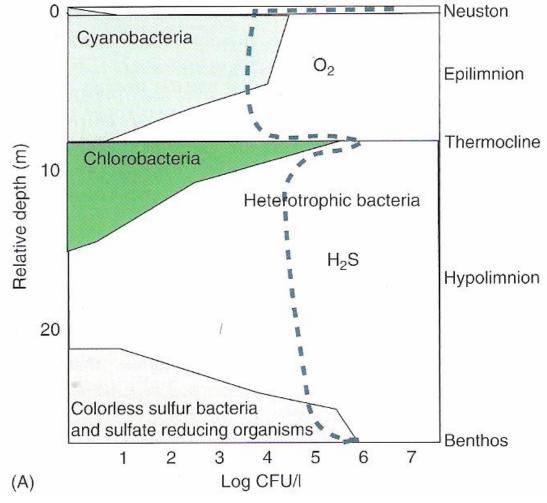


Idealized profiles of temperature and oxygen in a temperate region, eutrophic lake during the summer (A). Stratification is due to thermal warming of the upper layers in the summer months. Cooling of 'the upper layer in the fall and early winter breaks the mixing barrier and allows the sediment zone to be reoxygenated.

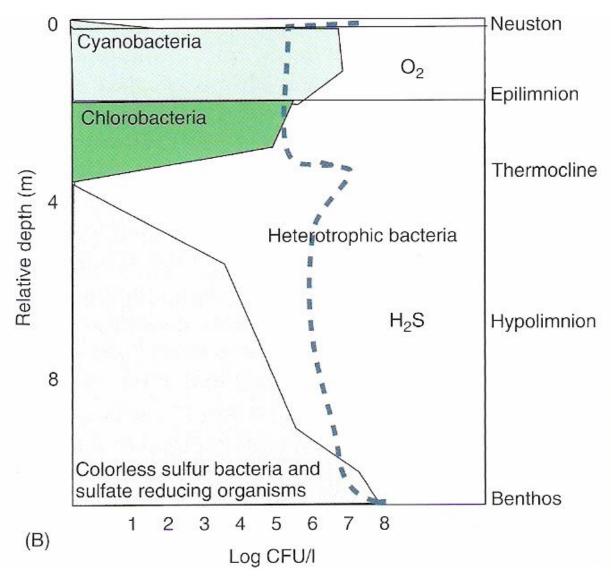


Idealized profiles of temperature and oxygen in a temperate region, eutrophic lake during the Winter (B). Stratification is due to thermal warming of the upper layers in the summer months. Cooling of 'the upper layer in the fall and early winter breaks the mixing barrier and allows the sediment zone to be reoxygenated.

#### **Microbial characteristics**



bacterial distribution in a typical oligotrophic lake. Notice especially the distribution and concentrations of the photosynthetic populations. Also note the lower concentration of heterotrophs in the upper zone, where cyanobacteria predominate. The large increase in the heterotrophic population between the epilimnion and the hypolimnion is related to the presence of a zone where organic matter accumulates. This area is known as a thermocline and is a zone where the sunlight-warmed surface water (less dense) and the deeper colder water (more dense) meet, forming a density gradient where organic matter accumulates.



Schematic representation of a typical eutrophic lake. The figure show the same groups of organisms as in A, indicating the localization and relative concentrations throughout the water column. Notice that both the photosynthetic and the heterotrophic populations are considerably higher in a eutrophic lake.

## 2. Brackish Water

Brackish water is a broad term used to describe water that is more saline than freshwater but less saline than true marine environments.

Often these are transitional areas between fresh and marine waters.

An estuary دلتا, which is the part of a river that meets the sea, is the best known example of brackish water.

Estuaries are highly variable environments because the salinity can change drastically over a relatively short distance.

Dramatic change can also occur at a given point in the estuary as a function of the time of day or season of the year.

In contrast, seasonal increases in freshwater due to rainfall or snowmelt will decrease the salinity at a given point in the estuary. The variation in salinity can range from 1.0 to 3.2%, with the average salinity of freshwater being 0.05%. In order to survive in these environments, microbes and plants in an estuary must be adapted to the fluctuations in salinity. Despite this, estuaries are very productive environments.

In general, estuarine primary production (10 to 45 mg carbon/m³/day) is not always enough to support the secondary populations.

Estuaries tend to be turbid because of the large amount of organic matter brought in by rivers and the mixing action of tides. As a result, light penetration is poor.

Numbers of primary producers are variable, ranging from 10<sup>0</sup> to 10<sup>7</sup> organisms/ml, and these populations also vary considerably in relation to depth and proximity to existing littoral zones.

Despite low primary productivity, because availability of substrate is not limited, heterotrophic activity is high, ranging from 150 to 230 mg carbon/m³/day.

Local runoff and organic carbon are brought in abundantly بكثرة by the rivers that flow into the estuaries.

In fact, the supply of nutrients can be so great that in many cases estuaries can actually become anoxic for whole seasons during the year. As a result of the steady and abundant carbon supply, numbers of secondary producers fall into a much narrower range from 10<sup>6</sup> to 10<sup>8</sup> organisms/ml.

Measured viral to bacteria ratios range from 0.4 to 50

## 3. Marine Water

Marine water is characterized by salinity between 3.3 and 3.7% and can range in depth up to 11,000 m in the deepest of ocean trenches.

Oceans are not static in size or shape; considerable mixing (especially of the surface layers) and movement are caused by the action of tides, currents, temperature upwelling, and winds.

Because the oceans are so expansive and their surface areas are so great, the effect of sunlight is important.

The ocean is divided into two zones:

The photic zone, through which light can penetrate

The lower aphotic zone

Light is able to penetrate to a depth of 200 m, depending on the turbidity of the water.

In coastal areas, where the amount of suspended particulate matter in the water is high, light may penetrate less than I m.

Other designations for classifying zones in marine environments are based on habitats.

## Four major habitats are important from a microbiological standpoint.

- At the surface of the sea (air-water interface) is the habitat referred to as the neuston.
- The pelagic zone is a broad term used to describe the water column or planktonic habitat.
- The pelagic habitat is subdivided on the basis of the precise depth in the water column.
- The habitat in the upper 100 m of the water column is known as the epipelagic zone (i.e., the photic zone).

A large proportion of the organisms in the epipelagic zone are photosynthetic.

Further depths are designated as mesopelagic, bathypelagic, and abyssopelagic habitats.

Finally, the benthopelagic zone (benthos) is the sea-sediment interface.

Apart from the pelagic zone and the neuston layer, the third major habitat is the epibiotic habitat, which refers to surfaces on which attached communities occur.

The fourth is the endobiotic habitat, which pertains to organisms found within the tissues of other larger organisms such as fish.

One interesting bacterium that can live endobiotically is *Vibrio fischeri*, an organism that uses luminescence\* and quorum sensing\* الضوئي والنصاب القانوني in its fascinating lifestyle

- \* **Luminescence:** is light that usually occurs at low temperatures, and is thus a form of. It can be caused by chemical reactions, electrical energy, or stress on a crystal.
- \* **Quorum sensing:** is a type of decision-making process used by decentralized groups to coordinate behavior. Many species of bacteria use quorum sensing to coordinate their gene expression according to the local density of their population. Similarly, some social insects use quorum sensing to make collective decisions about where to nest

## Food for the future

Spirulina is a cyanobacterium (phytoplankton) that has potential to serve as an alternative dietary protein source.